Combined Heat and Controlled Atmosphere Quarantine Treatments for Control of Western Cherry Fruit Fly in Sweet Cherries

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ABSTRACT Nonchemical quarantine treatments, using a combination of short duration high temperatures under low oxygen, elevated carbon dioxide atmospheric environment were developed to control western cherry fruit fly, *Rhagoletis indifferens* Curran, in sweet cherries, *Prunus avium* (L.). The two treatments developed use a chamber temperature of 45°C for 45 min and a chamber temperature of 47°C for 25 min, both under a 1% oxygen, 15% carbon dioxide, −2°C dew point environment. Both these treatments have been shown to provide control of all life stages of western cherry fruit fly while preserving commodity market quality. There was no definitive egg or larval stage, which was demonstrated to be the most tolerant to either controlled atmosphere temperature treatment system treatment. Efficacy tests for both treatments resulted in 100% mortality of >5,000 western cherry fruit flies in each treatment. These treatments may provide, with further study, quarantine security in exported sweet cherries where western cherry fruit fly is a quarantine concern and fumigation with methyl bromide is not desired.

KEY WORDS *Rhagoletis indifferens*, quarantine, Prunus avium, controlled atmosphere, heat treatment

Western cherry fruit fly, *Rhagoletis indifferens* Curran, is the principal pest of sweet cherries, *Prunus avium* (L.), shipped from the Pacific Northwest to other states and to other countries (CDFA 2005, NWHC 2005). At the packing house, there is a zero tolerance for the presence of western cherry fruit fly in sweet cherries, and orchards having detected western cherry fruit fly in harvested fruit are banned from the packing house (WAC 1968). Currently, sweet cherries are certified by visual inspection or fumigated with methyl bromide to ensure quarantine security (WAC 1968, FAO 1983; Moffitt et al. 1992; NWHC 2004a,b). However, methyl bromide fumigation is not in compliance with international and U.S. organic standards (MAFF 2000, USDA–AMS 2004). Development of organic compliant quarantine treatments for organic sweet cherries would aid in the economic development of organic producers in the United States.

The controlled atmosphere (CA) temperature treatment system (CATTS) (Neven and Mitcham 1996) combines the application of forced moist or vapor hot air under a CA (i.e., low oxygen, elevated carbon dioxide). This technology is similar to existing vapor and forced hot air treatment systems currently approved and in use for a number of commodities going into a number of countries worldwide (Armstrong 1994, Hallman and Armstrong 1994, USDA–APHIS–PPQ 2003). The difference with CATTS is the application of a modified atmosphere or CA. CA treatments reduce the time necessary for 100% kill of the pest compared with heat treatment alone (Neven and Mitcham 1996, Neven 2005); and by decreasing the duration of the treatment, a combination treatment of this type can often reduce adverse effects on fruit quality caused by heat treatment (Neven et al. 2001; Shellie et al. 2001; Yahia 2000a,b,c). Combination heat and CA treatment research has increased in recent years (Neven and Mitcham 1996; Shellie et al. 2001, Shellie et al. 1997, Whiting and Hoy 1997; Neven and Drake 1998, 2000; Yahia 2000a,b,c; Whiting et al. 1999; Neven et al. 2001; Heather et al. 2002; Neven 2004, 2005; Obenland et al. 2005), indicating the applicability of this technology for a wide range of fruit.

As with all quarantine treatments, it is important to identify the most tolerant infestive stage to a treatment, because that stage would be the target of all subsequent tests to demonstrate the efficacy of the new treatment. This article focuses on the identification of the most tolerant stage of western cherry fruit fly infesting sweet cherries to CATTS treatments and the development of treatment protocols to achieve postharvest control of this pest.

Materials and Methods

CATTS Treatments. The CATTS chamber (Techni-Systems, Chelan, WA) used in these studies has been described previously (Neven and Mitcham 1996). Treatment lugs (plastic box) used in these tests were standard vented bottom OnoPac (Hilo, HI) papaya

treatment lugs (38.1 by 53.3 by 15.2 cm). The bottom of the lugs was lined with nylon organdy that was secured in place with hot glue and duct tape to prevent larvae from dropping out of the lug during treatment. A layer of nylon organdy was also placed on the top of the lugs and secured in place with double stick tape, hot glue, or both to prevent exiting larvae from being blown into the chamber during treatment. Infested fruit were placed into the lugs, and three temperature probes were placed randomly under one surface of a fruit and into two fruit cores. The treatment chamber was run until treatment conditions for the test were achieved before loading the infested fruit. Treatment 1 consisted of a chamber temperature of 45°C, under 1% oxygen, 15% carbon dioxide environment with the dew point set to 2°C below the fruit surface temperature. Air speed was set at 2 m/s. Treatment 2 consisted of a chamber temperature of 47°C, with all other conditions the same as for treatment 1. Lugs of infested fruit were placed into the lug exchanger, which was part of the original CATTS system, and the lug exchanger was attached to the frame of the CATTS chamber. The lug exchanger was designed to be attached to the front of the CATTS chamber to minimize loss of atmospheric conditions during loading and unloading. The lug exchanger is a 156.85 by 73.66 by 45.72 cm (height × width × depth) unit that has four chambers (24.13 by 60.96 by 44.45 cm) that hold the treatment lugs and line up with the four doors of the CATTS chamber. The unit attaches to the front of the CATTS chamber with quick-release clamps. Compressible neoprene gaskets seal around the inner door frame unit which contains the sliding doors used for lug insertion. The lug exchanger was flushed with nitrogen for 2 min before inserting the lug into the chamber to help maintain CA levels in the CATTS chamber during exchanges. A sliding door was opened on the chamber and the lug inserted into the chamber. At that time, the temperature probe connector was attached to the junction inside the chamber to allow for monitoring of treatment temperatures. The door was closed immediately. Exchange of lugs normally took between 8 and 15 s. After the insertion of the lug into the chamber, the lug exchanger was detached from the chamber and the outer door sealed. The reverse process was used to retrieve lugs after treatment. All treated and control infested fruit were subjected to forced air cooling for 2 h in a 0°C walk-in cold room by placing a household box fan over the lug of fruit.

Most Tolerant Stage. Adult western cherry fruit fly were collected from various sites in central Washington during the 1999–2004 growing seasons. Flies were collected from cherry trees by using hand-held aspirators in the morning while either mating or ovipositing on the fruit. Approximately 200 pairs of western cherry fruit fly were placed into a 30.4-cm^3 (1-$foot^3$) screen cage and held at normal rearing conditions (23°C, 70% RH, and a photoperiod of 18:6 h [L:D] h) for up to 8 d to allow for oviposition. Approximately 30 mature 'Bing' sweet cherries were suspended in the cage by using fishing line and binder clips. Fresh fruit were placed in the cage every 2 d. Fruit were held at normal rearing conditions until the desired developmental stage was reached. Under these conditions, we determined that the egg stages were divided into 0–2-, 2–4-, 4–6-, 6–8-, and 8–10-d groups. First instars were allowed to develop for 12 d, whereas second and third instars were allowed to develop for 14 and 17 d, respectively.

Eight groups of 30 western cherry fruit fly-infested sweet cherries were placed into a subdivided treatment lug. Control fruit were handled in the same manner. Treatment 1 consisted of a chamber temperature of 45°C, under 1% oxygen, 15% carbon dioxide environment with the dew point set to 2°C below the fruit surface temperature. Air speed was set at 2 m/s. Treatment 2 consisted of a chamber temperature of 47°C, with all other conditions the same as for treatment 1. Time points for treatment 1 were 0, 10, 20, 30, and 40 min, whereas time points for treatment 2 were 0, 5, 10, 15, 20, and 25 min.

After CATTS treatment, treated and control fruit were forced air cooled at 0°C for 2 h. Treated and control fruit were held at normal rearing conditions for up to 30 d to allow for completion of development and exiting of the mature larvae from the fruit before pupation. Fruit were held in 10- by 15- by 10-cm plastic deli containers (Pactiv, Lake Forest, IL), on a 1-cm wire mesh, suspended over a 3-cm-deep pool of water. Larvae exiting the fruit were collected in the water and determined to have survived the treatment. The water below the cherries was examined daily for larvae.

Large-Scale Efficacy Tests. Naturally infested sweet cherries (with western cherry fruit fly) were collected from four sites in central Washington during the 2003 field season. Fruit were divided controls and treatments. Because infestation levels varied from site to site, the amount of larvae in a given weight of the untreated control was used as a basis to estimate the amount of larvae in the treatments. Two CATTS treatments of 45°C for 45 min and 47°C for 25 min under a 1% oxygen and 15% carbon dioxide, −2°C dew point environment with an air speed of 2 m/s were performed. Treatments and evaluations were conducted as described previously. Critical target temperatures for the 45°C CATTS treatment were that core temperature of the fruit reach 42°C within 7–9 min and reach a final core temperature of 44.5°C in 22–24 min. Critical target temperatures for the 47°C CATTS treatment were that fruit core temperatures reach 42°C within 7–9 min and reach a final core temperature of 45.5°C in 12–14 min (Fig. 1). Any treatments not achieving these requirements, or when the atmospheres did not meet minimum requirements (2% O_2, 10% CO_2), were not included in the study. After CATTS treatments, fruit were forced air cooled for 2 h at 0°C. Both control and treated infested fruit were placed in 49.5 by 36.0 by 12.4 cm Rubbermaid containers on top of a 1-cm square wire mesh with ≈4 cm of water in the bottom of the container. The control and treated fruit were held at normal rearing conditions (23°C, 70% RH, and a photoperiod of 18:6 [L:D]

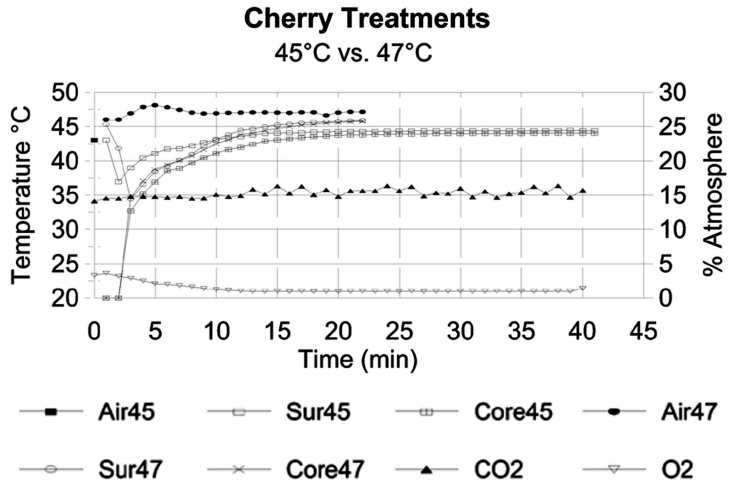

Fig. 1. Comparison of two CATTS treatment conditions for sweet cherries. One treatment is performed at a chamber temperature of 45°C (■) for a total of 45 min, whereas the other treatment is performed at a chamber temperature of 47°C (●) for a total of 25 min. Atmospheric conditions are indicated by ▲ (CO_2) and ▽ (O_2). Fruit Surface and core temperatures are denoted by □ (surface at 45°C), ■ (core at 45°C), ○ (surface at 47°C) and x (core at 47°C).

h) for up to 30 d to allow for completion of development and exiting of the mature larvae from the fruit before pupation. This longer hold time was added to accommodate any larvae or eggs whose development may have been delayed due to the effects of heat shock as a result of the treatment. The total number of western cherry fruit flies treated was determined by multiplying the number of western cherry fruit fly larvae collected per kilogram in the control fruit by the number of kilograms in the treatment.

Statistics. Probit analysis (Proc Probit, SAS Institute 2000) was used to determine the most tolerant stage. The time variable was transformed using the natural logarithm. Analysis of covariance (SAS Institute 2000) also was used to determine interaction effects in the most tolerant stage experiments. All mortality data were corrected for control mortality using Abbott's equation (Abbott 1925) and arcsine transformed before statistical analyses.

Results and Discussion

Most Tolerant Stage. Determination of the most tolerant stage of western cherry fruit fly was inconclusive when comparing CATTS treatment effects. The algorithms converged for all probit analyses (Tables 1 and 2). Although probits were calculated for all life stages, confidence limits were not calculated for the 2–4-d-old eggs for the 45°C CATTS treatment and for the 0–2-d-old eggs for the 47°C CATTS treatment (Tables 1 and 2; Fig. 2). This is due to the high amount of variation in survivorship for these stages in the two treatments. The overlapping confidence intervals made the identification of the most tolerant infestive

Table 1. Probit analysis of the larval and egg stages of western cherry fruit fly after a CATTS treatment at 45°C

Stage	LT_{50} (min)	95% CL (min)	LT_{90} (min)	95% CL (min)	df	Intercept χ^2	Log_{10} time χ^2	Intercept $Pr > \chi^2$	Log_{10} time $Pr > \chi^2$
First instar[a]	8.80	6.8–10.3	23.98	20.8–28.5	3	45.25	81.92	<0.0001	<0.0001
Second instar[b]	8.08	0.51–15.62	29.37	15.16–281.22	3	8.62	17.19	0.0033	<0.0001
Third instar[c]	7.16	1.93–12.28	25.45	15.11–69.21	3	14.15	30.63	0.0002	<0.0001
0–2 d egg[d]	8.99	6.95–10.91	31.65	26.30–40.31	3	46.05	84.10	<0.0001	<0.0001
2–4 d egg[e]	13.30	ND	36.17	ND	3	4.53	6.40	0.0333	0.0114
4–6 d egg[f]	9.46	6.67–12.13	38.49	29.55–56.69	3	29.23	51.98	<0.0001	<0.0001
6–8 d egg[g]	8.21	6.23–10.05	26.35	22.08–32.92	3	40.94	78.99	<0.0001	<0.0001
8–10 d egg[h]	6.16	0.89–11.91	21.08	10.73–75.55	3	9.69	23.47	0.0019	<0.0001

Algorithms converged for all probits. ND, not determined.
[a] Fudicial limits calculated using a t value of 1.96.
[b] Variance and covariance multiplied by heterogeneity factor H = 6.0581. Fiducial limits calculated by t value of 3.18.
[c] Variance and covariance multiplied by heterogeneity factor H = 4.8085. Fiducial limits calculated by t value of 3.18.
[d] Fudicial limits calculated using a t value of 1.96.
[e] Variance and covariance multiplied by heterogeneity factor H = 5.4452. Fiducial limits calculated by t value of 3.18.
[f] Fudicial limits calculated using a t value of 1.96.
[g] Fudicial limits calculated using a t value of 1.96.
[h] Variance and covariance multiplied by heterogeneity factor H = 7.6128. Fiducial limits calculated by t value of 3.18.

Table 2. Probit analysis of the larval and egg stages of western cherry fruit fly after a CATTS treatment at 47°C

Stage	LT_{50} (min)	95% CL (min)	LT_{90} (min)	95% CL (min)	df	Intercept χ^2	Log_{10} time χ^2	Intercept Pr > χ^2	Log_{10} time Pr > χ^2
First instar[a]	8.31	1.5–13.2	28.4	17.8–178.5	3	11.33	19.54	0.0008	<0.0001
Second instar[b]	6.67	2.19–10.24	20.68	14.01–43.41	3	15.50	32.21	<0.0001	<0.0001
Third instar[c]	9.15	7.05–10.86	27.79	23.3–36.42	3	31.48	50.30	<0.0001	<0.0001
0–2 d egg[d]	13.80	ND	65.66	ND	3	7.78	9.01	0.0053	0.0027
2–4 d egg[e]	9.15	1.79–13.89	30.47	19.54–228.01	3	11.86	18.86	0.0006	<0.0001
4–6 d egg[f]	11.62	7.88–14.33	37.10	27.32–76.71	3	14.03	18.72	0.0002	<0.0001
6–8 d egg[g]	7.18	5.25–8.90	23.55	19.43–30.63	3	29.61	57.67	<0.0001	<0.0001
8–10 d egg[h]	5.83	0.05–11.65	18.65	8.62–222.31	3	6.08	14.28	0.0136	0.0002

Algorithms converged for all probits. Time was converted to log_{10} to run probits and then converted back to minutes to report LT and 95% CL. ND, not determined.

[a] Variance and covariance multiplied by heterogeneity factor H = 3.1674. Fiducial limits calculated by *t* value of 3.18.
[b] Variance and covariance multiplied by heterogeneity factor H = 2.938. Fiducial limits calculated by *t* value of 3.18.
[c] Fiducial limits calculated by *t* value of 1.96.
[d] Variance and covariance multiplied by heterogeneity factor H = 2.749. Fiducial limits calculated by *t* value of 3.18.
[e] Variance and covariance multiplied by heterogeneity factor H = 2.5327. Fiducial limits calculated by *t* value of 3.18.
[f] Fudicial limits calculated using a *t* value of 1.96.
[g] Fudicial limits calculated using a *t* value of 1.96.
[h] Variance and covariance multiplied by heterogeneity factor H = 10.643. Fiducial limits calculated by *t* value of 3.18.

stage insuperable. The addition of the CA nearly eliminated the thermal response or acclimation of the different infestive stages (Figs. 3 and 4). CA temperature treatments prevent thermal acclimation by addition of a low oxygen, elevated carbon dioxide environment along with the heat treatment (Neven 2004). Under CA conditions, the production of heat shock proteins by insects is inhibited, indicating a compromise of the acclimation process (L.N., unpublished data). When mortality of each stage in the two treatments was plotted (Figs. 3 and 4), all stages followed a similar curve, and no stage emerged as being most tolerant when we compared mortalities with SEMs. The egg stages seem to be marginally more tolerant, but the difference was not statistically significant.

Efficacy Tests. Both CATTS treatments effectively controlled all stages of western cherry fruit fly from naturally infested fruit (Table 3). We estimate that all

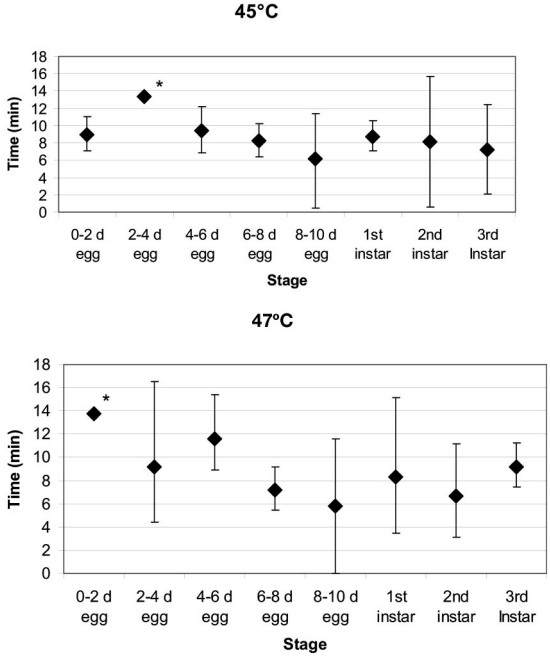

Fig. 2. LT_{50} with 95% CI for five egg and three larval stages of western cherry fruit fly in sweet cherries subjected to CATTS treatments of 45°C (A) and 47°C (B) under a 1% O_2, 15% CO_2 atmosphere. The asterisk (*) indicates samples where the 95% CI could not be calculated.

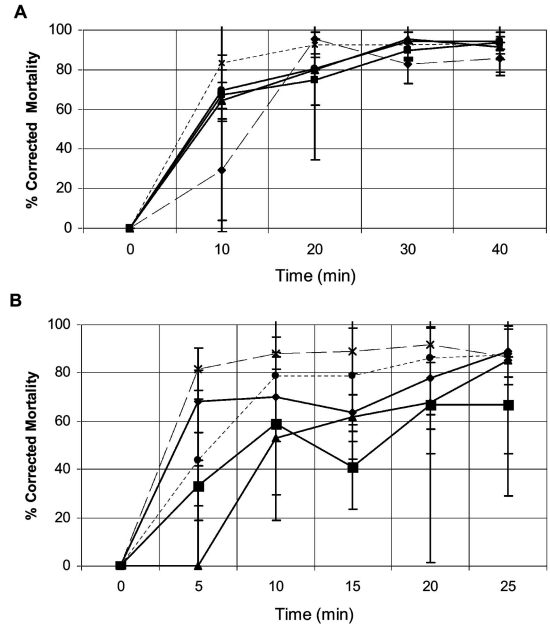

Fig. 3. Mortality of the five egg stages of western cherry fruit fly in sweet cherries subjected to nonoptimal CATTS treatments of 45°C (A) and 47°C (B) under a 1% O_2, 15% CO_2 atmosphere where core temperatures did not reach 42°C in 9 min to allow for optimal variability and survivorship at the last time points.

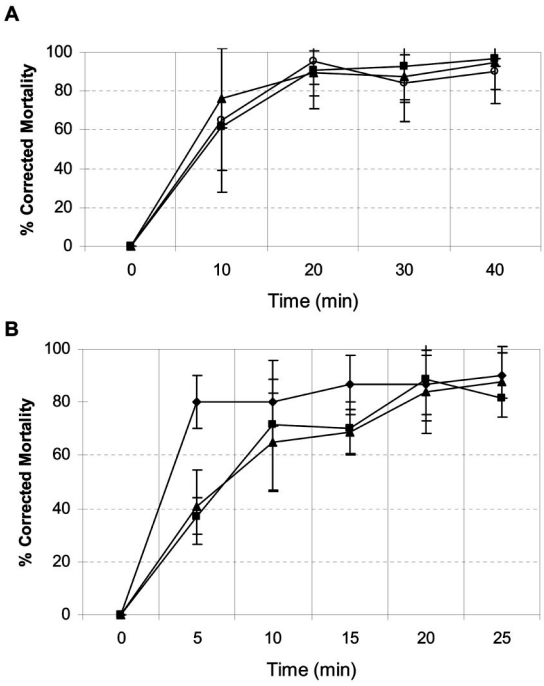

Fig. 4. Mortality of the three larval stages of western cherry fruit fly in sweet cherries subjected to nonoptimal CATTS treatments of 45°C (A) and 47°C (B) under a 1% O_2, 15% CO_2 atmosphere where core temperatures did not reach 42°C in 9 min to allow for optimal variability in survivorship at the last time points.

egg and larval stages were represented in the infested fruit because we observed pupation from the control fruit for 3 wk after treatment. We estimated that we treated and killed 6,315 larvae in the 45°C treatment and 5,800 larvae in the 47°C treatment. Efficacy trials generally require killing 5,000 of the most tolerant stage with zero survivors. We exceeded this requirement in both tests.

These treatments were the same as those developed to control codling moth, *Cydia pomonella* (L.), in sweet cherries (Neven 2005). We found that we could control all life stages of codling moth with CATTS treatments of 45 and 47°C for 25 and 45 min with minimal effect on fruit quality (Neven and Drake 2000, Neven 2005).

These studies indicate that CATTS can be effective in achieving control of western cherry fruit fly in sweet cherries. Previous research has demonstrated that CATTS treatments of sweet cherries provides acceptable market quality with up to 3 wk of shelf life (Neven and Drake 1998, Neven and Drake 2000, Neven et al. 2001, Shellie et al. 2001). These treatments show great promise as both an alternative to methyl bromide fumigation for conventional and a new treatment for organically grown sweet cherries.

Table 3. Results from CATTS efficacy tests of all life stages of western cherry fruit fly in sweet cherries

Treatment	Duration (min)	Control (*n*)	Treatment (*n*)	% survivorship
Control	0	712		100.0
45°C	45	110	6315	0.0
47°C	25	602	5800	0.0

Treatments were 45°C for 45 min and 47°C for 25 min under a 1% O_2, 15% CO_2 atmosphere with a −2°C dew point and 2.0 m/s air speed.

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